Diastereoselective Amplification of an Induced-Fit Receptor from a Dynamic Combinatorial Library

Corbett, Peter T.; Tong, Lok H.; Sanders, Jeremy K.M.; Otto, Sijbren

Published in:
Journal of the American Chemical Society

DOI:
10.1021/ja050790i

Publication date:
2005

Supporting Information for:

Diastereoselective Amplification of an Induced-Fit Receptor from a Dynamic Combinatorial Library

*Peter T. Corbett, Lok H. Tong, Jeremy K. M. Sanders and Sijbren Otto**

Journal of the American Chemical Society

Contents:

Materials and methods

Dynamic Combinatorial Libraries were constructed by dissolving **1** in water, and adjusting the pH to 8 using NaOH and HCl. The resulting solutions (5mM unless otherwise stated) were allowed to equilibrate for at least 3 weeks, either in the absence or presence of the NMe_4I (5mM), by stirring as 1mL aliquots in closed 2mL HPLC vials.

The DCL in Figure 1 was made using a different method: a 10mM solution of **1** and **5** (1:1) was made in a 100mM pH8 phosphate buffer. The oxidation of the thiols was catalysed using 0.1mM $CuCl_2$, and the library was analysed after 1 week.

Host 4 was isolated by preparative HPLC from a DCL prepared using 10mM **1** and 10.7mM NMe_4I. Aliquots of 500µl of the DCL solution were chromatographed (Nucleodur C_{18} preparative column (25.0cm x 2.1cm, 100Å, 5µm) with a Nucleodur C_{18} guard column (5.0cm x 2.1cm, 100Å, 5µm), 20mL/min 55:45:0.1 acetonitrile/water/trifluoroacetic acid, 45^{o}C, retention time 24-27min). The collected fractions from 23 injections were dried *in vacuo*, and redissolved in 5mL 1:1 acetonitrile/water, and rechromatographed as before in one single injection. This product was again dried *in vacuo* and redissolved in 3mL 50mM borate buffer, and separated into two 1.5mL aliquots. 100µl 2M HCl was added to each aliquot, and the resultant suspensions were centrifuged. The pellets were washed twice by addition of 500µl 40mM HCl, resuspension and recentrifugation. The final pellets were dried *in vacuo* overnight (0.91mg each).

Analyses of 4

Mass (ESI-MS, -ve ion): *m/z* = 1414.77.

Anal. Calcd for **4**·$7H_2O$ ($C_{72}H_{54}O_{23}S_8$) C, 56.02; H, 3.53. Found: C, 56.31; H, 3.34.

4: **^{1}H NMR 500MHz CD_3OD 300K**: 7.50 (8H, s, ArH), 7.28 (8H, s, ArH), 7.07 (8H, s, ArH), 5.92 (8H, s, CH).

4 + NMe_4I: ^{1}H NMR 500MHz D_2O (pD 8.6, 50mM K^+/borate) 300K: 8.00 (4H, s, ArH), 7.94 (4H, s, ArH), 7.64 (4H, d (poorly resolved) , ArH), 7.57 (4H, d (poorly resolved) , ArH), 7.46 (4H, d, *J* = 6.5Hz, ArH), 6.49 (4H, d (poorly resolved), ArH), 5.59 (4H, s, CH), 5.48 (4H, s, CH), 3.90 (~60H, unbound NMe_4^+), -1.25 (12H, bound NMe_4^+).

4 + NMe_4I: ^{1}H NMR 500MHz D_2O (pD 8.6, 50mM K^+/borate) 360K: 8.53 (8H, s, ArH), 8.12 (8H, s, ArH), 7.77(8H, broad s, ArH), 6.17 (8H, s, CH), 3.26 (v. broad s).

HPLC Methods

HPLC analyses were carried out on a Hewlett Packard 1050 system coupled to a UV analyzer, set to 320nm. The data were processed using HP Chemstation software. Separations were achieved using Waters Symmetry C_{18} columns (25.0 cm × 4.6 mm, 5

μm particle size). For Fig. 1a,b, the following gradient was used at 1mL/min, at ambient temperature, with 10μl injections:

Time (minutes)	**% (Acetonitrile + 0.1% trifluoroacetic acid)**	**% (Water + 0.1% trifluoroacetic acid)**
0	5	95
30	95	5
35	95	5
36	5	95
50	5	95

For Fig. 1c,d, Fig. SI3 and Fig. SI4, an isocratic mobile phase was used at 1mL/min, consisting of acetonitrile, water and trifluoroacetic acid in the ratio of 55:45:0.1. The column was heated to 45^{o}C. 2μl injections were used.

ITC Analysis

The analysis of the binding between **4** and NMe_4I was conducted twice, and average values were taken for the thermodynamic parameters. Solutions of NMe_4I (1.02mM and 0.496mM in 10mM borate puffer pH 9) were titrated into solutions of **4** (0.0786mM in 10mM borate puffer pH9). Analysis of the binding curves gave a poor fit to a simple 1:1 mode of binding, with a curve shape as shown in Fig. SI1. At least two hypotheses could explain the observed binding curve – an initial 2:1 binding mode, which is broken up by the addition of further guest, is one explanation. Alternatively, and more likely, the host could be forming non-covalent aggregates in the absence of guest, which are broken up (endothermically) by the addition of the guest. To get an approximate value for the 1:1 binding constant in the system, the initial part of the binding curve was discarded, and curve-fitting was carried out on the rest of the trace, as shown in Fig. SI2.

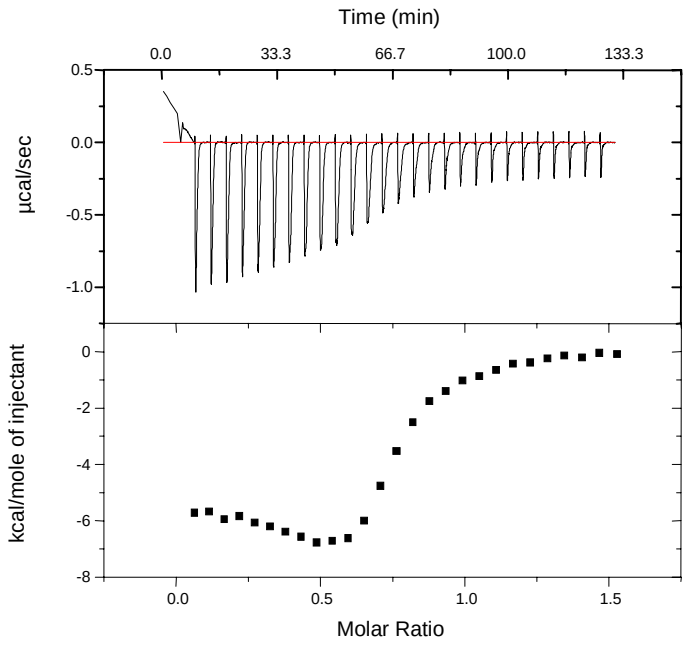

Figure SI1: ITC trace for the titration of NMe4I into **4**.

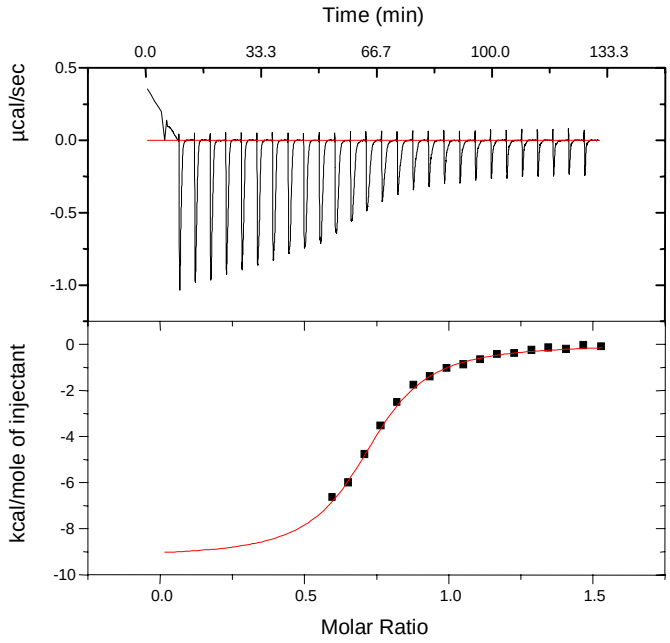

Figure SI2: ITC trace for the titration of NMe_4I into **4**, showing the data points used for fitting.

Assignment of 2 and 3

A sample of the **2** was isolated by preparative HPLC, and characterized:

^{1}H NMR 500MHz CD_3OD 300K: 7.57 (2H, s, ArH), 7.47 (2H, s, ArH), 7.38 (2H, s, ArH), 7.26 (2H, d, *J* = 7.6 Hz, ArH), 7.20 (4H, m, ArH), 7.01 (2H, d, *J* = 7.9 Hz, ArH), 6.89 (4H, m, ArH), 5.82 (2H, s, CH), 5.81 (2H, s, CH), 5.72 (2H, s, CH).

A mixture of **2** and **3** has previously been characterized by NMR: Otto, S.; Furlan, R. L. E.; Sanders, J. K. M. *Science* **2002**, *297*, 590-593.

Re-equilibration experiments

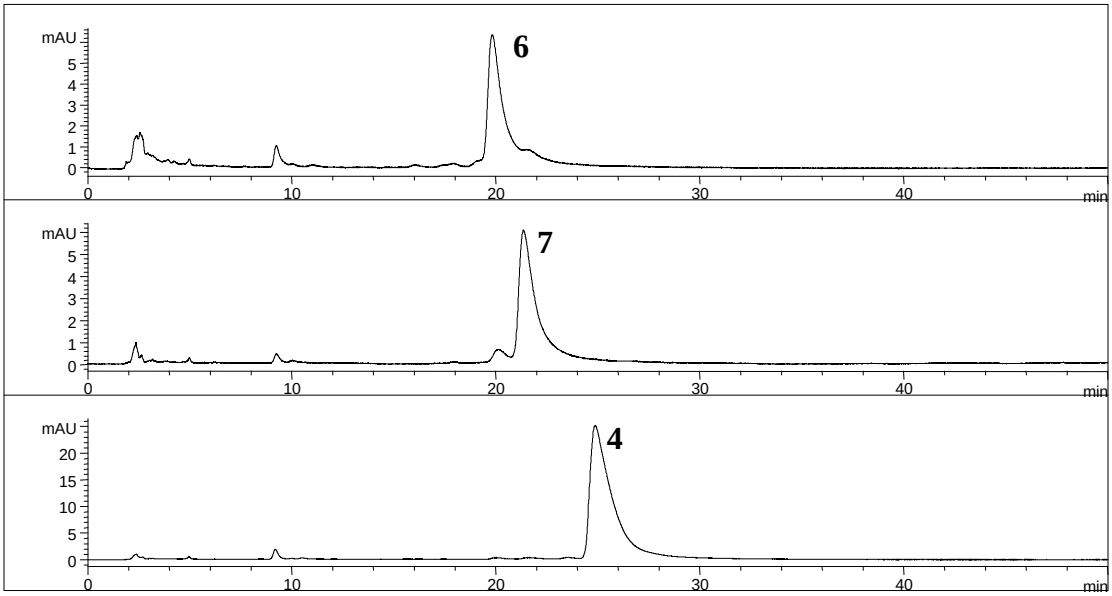

Figure SI3: HPLC analyses of samples of tetramers, after dissolution in 10mM borate buffer pH 9.

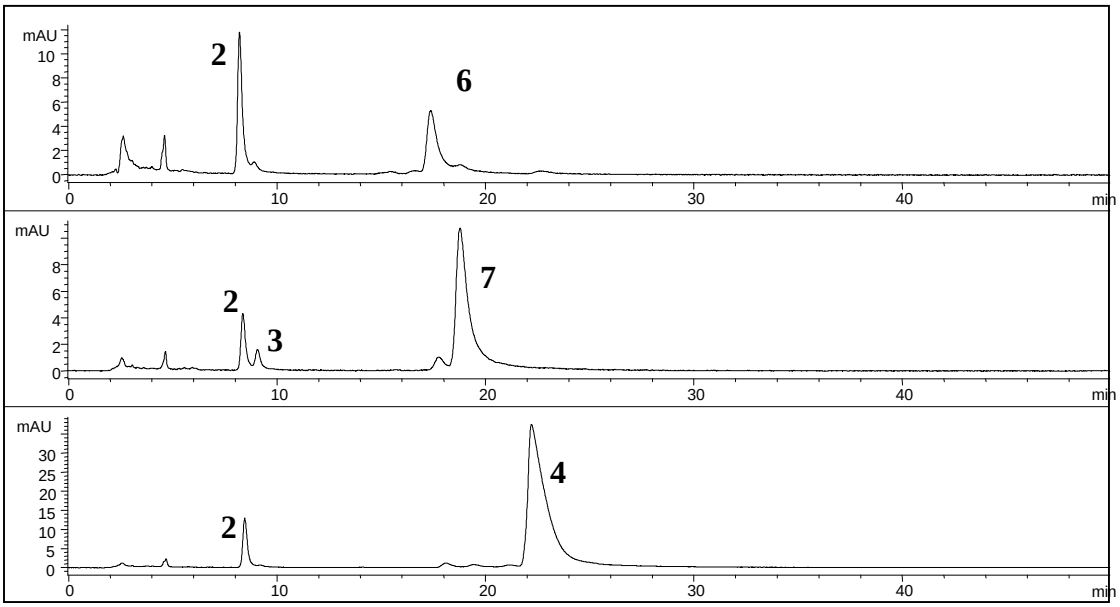

Figure SI4: HPLC analyses of samples of tetramers, after 7 days of re-equilibration in buffer.